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VOLUME II NUMBER 6

JOURNAL OF BACTERIOLOGY

OFFICIAL ORGAN OF THE SOCIETY OF AMERICAN BACTERIOLOGISTS

NOVEMBER, 1917

EDITOR-IN-CHIEF
C.-E. A. WINSLOW

It is characteristic of Science and Progress that they continually open new fields to our vision.—PASTEUR

PUBLISHED BIMONTHLY
WILLIAMS & WILKINS COMPANY
BALTIMORE, U. S. A.

THE CAMBRIDGE UNIVERSITY PRESS
FETTER LANE, LONDON, E. C.

Entered as second-class matter April 21, 1916, at the Post Office at Baltimore, Maryland, under the Act of March 3, 1879

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EARLY INSTRUCTORS IN BACTERIOLOGY IN THE UNITED STATES

DAVID H. BERGEY

University of Pennsylvania

I have collected from various sources information concerning the early instructors in bacteriology in the United States. I have been especially fortunate in that it has been possible to obtain much of the information from the teachers themselves, though in many instances my informants were obliged to rely, in part, on their memory as to the dates when attention commenced to be directed to the bacteria and instruction was offered to students. I have attempted to arrange, in proper sequence, the order in which these several teachers began their work, but I may have failed to give the exact order. Other names might have been included in this list had it been possible to obtain the desired information. Following the publication by Pasteur of the results of his investigations on the relations of the bacteria to fermentation and to disease, several scientists in this country took up, independently, the study of bacteria by Pasteur's methods. These men were interested in the bacteria from either the broad biological standpoint or from the standpoint of pathology.

Probably the first name in the list of early teachers is that of the late Dr. T. J. Burrill who introduced the study of the bacteria into his course on the fungi during the "seventies." He discovered the organism of pear blight in 1879, and in the following years conducted, extensive inoculation experiments with this organism on a large orchard of young pear trees, thereby definitely establishing the etiological relation of the organism to the disease. In 1882 Dr. Burrill published a pamphlet entitled "Bacteria," in which he gives a discussion of the morphology, functions, classification and methods of study—the

methods of study being those of the French school. The classification of the bacteria follows that of Ferdinand Cohn. There is also included a brief description of the bacteria that were then identified. It was not, however, until 1892 that a course in general bacteriology was organized at the University of Illinois aside from the work which had been given in the course on the fungi.

Probably the next in order of seniority is the late Surgeon General George M. Sternberg, whose investigations on the causation of yellow fever, malaria, syphilis and other diseases are well known, who discovered the pneumococcus in normal sputum, and laid the foundations for our knowledge of the value of a large number of chemical substances as disinfectants. Dr. Sternberg translated Magnin's work on the "Bacteria" in 1880 and issued an enlarged and revised edition in 1884. In 1892 he published his large Manual of Bacteriology, and in 1896 his Text Book of Bacteriology.

The next in order is probably Dr. William H. Welch who from 1878 on was interested in the bacteria and their relation to disease, demonstrating them in sections of tissues to his classes in pathology at the Bellevue Hospital Medical College; and after Koch's discovery of the tubercle bacillus the study of this organism in sputum and tissues became an important part of the instruction. On returning from Europe in 1885 Dr. Welch became the head of the Pathological Institute at Johns Hopkins University, and had for his assistant in the instruction in bacteriology Dr. A. C. Abbott who had been Dr. Sternberg's assistant in the Biological Laboratory the previous year. Later on Dr. George H. F. Nuttall also became his assistant, and was associated with Dr. Welch in the discovery and study of the "gas" bacillus.

About the same time that Sternberg and Welch were directing their attention to the bacteria, Dr. T. Mitchel Prudden at the College of Physicians and Surgeons in New York also taught the staining of bacteria in sections of tissues and in sputum to his students in pathology, and commenced the cultivation of bacteria on solid media about 1883. Dr. Prudden, aside from

his interest in the pathological action of bacteria, also very early interested himself in the relation of bacteria to air, water, and ice, which were subjected to critical study, the results forming the basis of valuable monographs.

Beginning about 1879, Dr. D. E. Salmon commenced his important studies on the relation of bacteria to animal diseases in the Bureau of Animal Industry at Washington, and while Dr. Salmon is not known generally as a teacher of bacteriology, there is every evidence that he was the instructor of assistants in the Bureau and was the leading inspiration for many of the early discoveries made by the Bureau staff; notably the epoch making work which he did in association with Dr. Theobald Smith on Texas cattle fever, work which in a broad sense can be included here, even though the organism responsible for the disease is not a bacterium, but a protozoon.

In the group of instructors in pathology in medical schools who were interested in the relation of the bacteria to disease was the late Dr. Henry Formad at the University of Pennsylvania. In the autumn of 1882, Dr. Formad, who had visited and studied in Koch's laboratory during the summer, taught in class the method of diagnosing tuberculosis by staining the bacilli in sputum, and demonstrated various bacteria and the methods of cultivating them. In the autumn of 1883, after another visit to Koch's laboratory, Dr. Formad gave more extended demonstrations to his class of the apparatus and methods that were then in use by Koch in his laboratory. Dr. Formad made an investigation into the cause of diphtheria in an outbreak occurring in the state of Michigan, being associated in this work with Dr. Horatio C. Wood. Unfortunately Dr. Formad's inadequate training led him to erroneous conclusions with regard to the cause of diphtheria. The diphtheria organism seems to have escaped him, and he attributed the disease to a micrococcus. My first introduction to the bacteria was obtained in Dr. Formad's laboratory and from his lectures on the germ theory of disease.

Dr. W. T. Councilman, though not a bacteriologist, belonged in that group of early teachers of pathology who demonstrated

the bacteria in tissues and in secretions as a part of the routine course in pathological histology, and in this connection he became the director of students who later devoted their lives to a study of the bacteria.

Dr. A. C. Abbott became Dr. Sternberg's assistant at Johns Hopkins University in 1884, and assisted in his extensive studies on the value of chemical disinfectants, thus receiving his first training in bacteriology from Dr. Sternberg. In the spring of 1885, when Dr. Welch was made the director of the Pathological Institute, Dr. Abbott became his assistant as instructor in bacteriology and commenced to use the improved methods which Dr. Welch had learned in Koch's laboratory. Dr. Abbott had as his first student, in the spring of 1885, Dr. W. D. Booker, of Baltimore, who conducted an important investigation on the "summer complaints" of children; and shortly afterwards he also had as his student Dr. George H. F. Nuttall, now of Cambridge, England. Dr. Welch and Dr. Abbott were the first in this country to confirm the etiological relation of the diphtheria organism to the disease diphtheria, which had been announced from the German laboratories. When the Laboratory of Hygiene, University of Pennsylvania, was opened in 1892 under the direction of Dr. John S. Billings, Dr. Abbott became the first assistant, and a course in bacteriology was commenced at once. This course has been given to graduates in medicine and to other qualified students by different assistants in the laboratory under the direction of Dr. Abbott, and has been continued to the present time. In addition to this, all the instruction in bacteriology to the medical students has been given by the laboratory staff since 1896, and for some years both dental and veterinary students also received their instruction in bacteriology in the Laboratory of Hygiene. In 1892 Dr. Abbott published his Principles of Bacteriology, and this popular and valuable text book is now in its ninth edition.

Dr. Hermann M. Biggs took charge of the Carnegie Laboratory, at that time attached to Bellevue Hospital Medical College, in March, 1885, and began instruction in bacteriology to a class of graduate and undergraduate medical students.

The first formal course in bacteriology at the University of Wisconsin was given by Dr. E. A. Birge in the year 1885–1886. Some bacteriological apparatus and supplies had been purchased by the University at the request of Dr. William Trelease, and it was this apparatus which was utilized by Dr. Birge in this early course of instruction. Although Dr. Birge has maintained his interest in general biology, he has inspired some of our noted bacteriologists who studied under him, notably, Dr. H. L. Russell.

Dr. T. M. Cheesman began the first systematic instruction in bacteriological technique in Dr. Prudden's laboratory in the winter of 1885–1886. In the autumn of 1887 in the then new buildings of the College of Physicians and Surgeons on Fifty-ninth Street, systematic courses in practical bacteriology were offered to graduates in medicine and other technically qualified students.

Dr. John E. Weeks, who had spent the winter of 1884–1885 in Berlin studying pathology and bacteriology, entered the Ophthalmic and Aural Institute, 46 East Twelfth Street, as an interne. Dr. Herman Knapp returned from Europe in May, 1885, bringing with him appliances for bacteriologic and laboratory work, and asked Dr. Weeks to give a course in bacteriology. About November 1, 1885, a course, consisting of twenty lessons, was commenced at the Institute. This course was given to graduates in medicine only, and was repeated throughout the winters of 1885–1886 and 1886–1887.

Dr. Harold C. Ernst gave a course of six lectures on bacteriology at the Harvard Medical School in the fall of 1885. This was introductory to the course in general pathology given by Dr. R. H. Fitz.

Dr. Theobald Smith began a short course of lectures on hygiene which was largely bacteriological in the Medical Department of what is now the George Washington University in 1886. In 1894 he began to give laboratory instruction in bacteriology.

Beginning with the year 1886, Dr. L. H. Pammel began to direct the attention of the students in the Veterinary School of the

Iowa State College of Agriculture to the bacteria as a part of the course in cryptogamic botany. In the spring of the year 1889, Dr. Pammel began a general course in bacteriology to the senior veterinary students. This course was also offered to students in home economics.

Dr. Bayard Holmes gave a course in bacteriology to a class of three students in the Chicago Medical College (now Northwestern Medical School) during the spring of 1888, and in the spring of 1889 he became professor of bacteriology in the Postgraduate Medical School of Chicago. In July, 1889, he became secretary of the College of Physicians and Surgeons in Chicago and professor of bacteriology and surgical pathology, and began a systematic course in bacteriology, consisting of lectures and laboratory exercises, with a class of sixty students.

Dr. Victor C. Vaughn and Dr. F. G. Novy gave their first formal course in bacteriology in the winter of 1889, in the newly established Hygienic Laboratory of the University of Michigan. The course was elective, and was taken by students from the Medical and Literary Departments.

Dr. H. W. Conn first became interested in bacteria after hearing Dr. Farlow lecture in Boston in the year 1880 on "Low Forms of Plant Life," and had his first introduction to the bacteria at Johns Hopkins University under Dr. Councilman in 1881. Dr. Conn commenced to teach bacteriology about the year 1889, and his interest, aside from the broader biological significance of the bacteria, has been directed principally to the activities of the bacteria of milk and soil in their bearing on agriculture.

Dr. W. T. Sedgwick began the first formal course in bacteriology at the Massachusetts Institute of Technology during the academic year, 1888–1889.

Dr. Joseph MacFarland was appointed lecturer in bacteriology in the Medical School of the University of Pennsylvania in 1892, and gave a course of lectures with some laboratory exercises to the second year medical students. The course consisted in making hay infusions and studying the organisms that grew in them. Bacteriological studies of saliva, urine and sputum

were also made. The students were taught the ordinary methods of cultivation and staining, special attention being given to the staining of the tubercle bacillus.

Dr. William H. Park became professor of bacteriology at the Bellevue Hospital Medical College in 1895, when the first real teaching of bacteriology, as such, was begun by him.

From these simple beginnings the teaching of bacteriology has come in a comparatively brief time to play a very important part in the scientific education of many persons, and bacteriology is today being taught in a large number of educational institutions in this country. Courses are given, not only in elementary bacteriology to general science students but to students in domestic science, agriculture, dairying, water and sewage purification, public hygiene and sanitation, medicine, dentistry, veterinary medicine, pharmacy, brewing and fermentation industries, food production and preservation and plant pathology.

STUDIES ON THE NOMENCLATURE AND CLASSIFICATION OF THE BACTERIA

IV. SUBGROUPS AND GENERA OF THE COCCACEAE

R. E. BUCHANAN

From the Bacteriological Laboratories, Iowa State College, Ames, Iowa

Family 1. **Coccaceae** Zopf, 1884, p. 45, emended Migula 1894

Synonyms:

Sphaerobacteria Cohn, 1872, p. 146
Coccaceen Zopf, 1884, p. 45
Coccogenae Trevisan, 1885, p. 92
Coccacei Schroeter, 1886, p. 143
Coccobacteria Schroeter, 1886, p. 143
Sphaerobacteries Maggi, 1886, p. 81
Kokkaceen Hueppe, 1886
Coccacees Mace, 1897, p. 334

Cells usually spherical, sometimes somewhat elongated just before division, or when occurring in pairs or groups cells may be somewhat flattened on sides in contact. Never containing granules of sulphur, nor with bacteriopurpurin. Many species develop a pigment. Some species are motile by means of flagella. Cells frequently remaining in groups after division, forming chains, regular packets or irregular masses. Spore production doubtful, having been reported for but one species. Pseudoplasmodium never produced. Growth energy not secured by the oxidation of ammonia or nitrites.

The following names have been applied to groups designated as subfamilies, tribes and subtribes of the Coccaceae.

Ascococceae De Toni and Trevisan, 1889, p. 1037
Eu-Ascococceae De Toni and Trevisan, 1889, p. 1037
Gaffkyeae De Toni and Trevisan, 1889, p. 1042

Micrococceae De Toni and Trevisan, 1889, p. 1067
Sarcineae Trevisan, 1879, p. 136
Mycococcaceae Hansgirg, 1888, p. 266
Eucoccaceae Hansgirg, 1888, p. 267
Cystococcaceae Hansgirg, 1888, p. 266
Streptococceae De Toni and Trevisan, 1889, p. 1051
Homococcaceae Fischer, 1897, p. 32
Allococcaceae Fischer, 1903, p. 59
Metacoccaceae Winslow and Rogers, 1905, p. 669
Paracoccaceae Winslow and Rogers, 1905, p. 669

It is evident that, while many generic names have been proposed for cocci, very few authors have defined subfamilies, tribes or subtribes. Apparently the first effort was that of De Toni and Trevisan (1889, p. 1035). The following key to their tribes and subtribes indicates their grouping.

It will be noted that the chief emphasis throughout the classification is placed upon the development of cysts and capsules and upon cell groupings.

Key to Tribes of Coccogenae. De Toni and Trevisan

A. Cocci associated in families in bladder-like, gelatinous masses or cysts. Tribe I. *Ascococceae*
I. Cocci separated from each other in the mucus of the matrix.
a. Cocci destitute of special cysts, in families, in a common cyst. Subtribe I. *Eu-Ascococceae*
b. Cocci borne in special cysts. Common cysts absent. Subtribe II. *Gaffkyeae*
II. Cocci loosely united in a mucous matrix. Cysts generally delicate at length breaking apart. No special cysts. Subtribe III. *Amoebobacterieae*
B. Cocci associated in families of one or many layers, surrounded by a more or less evident mucous matrix. . . . . Tribe II. *Sarcineae*
C. Cocci in moniliform chains. Large arthrospores developing in the filaments or at the tips. . . . . Tribe III. *Streptococceae*
D. Cocci not in capsular cysts or sheaths, nor in moniliform chains. Small endospores in the cells. . . . . Tribe IV. *Micrococceae*

The classification of Winslow and Rogers (1905) separates the *Coccaceae* into two subfamilies, *Metacoccaceae* and *Paracoccaceae*. The subfamily *Metacoccaceae* is defined to include

the genera *Micrococcus*, *Sarcina* and *Rhodococcus*. The description given (1905, p. 669) is as follows: "Facultative parasites or saprophytes. Thrive best under aërobic conditions. Grow well on artificial media, producing abundant surface growths. Planes of fission often at right angles; cells aggregated in groups, packets or zoogloea masses." In later descriptions (1906, p. 845, and 1908, p. 258) they have added "Generally decolorize by Gram. Pigment yellow or red."

The subfamily *Paracoccaceae* (1905, p. 669) is defined to contain the genera *Diplococcus*, *Ascococcus*, *Streptococcus*, *Albococcus* and *Aurococcus*. The description is: "Parasites (thriving only, or best, on or in the animal body), thrive well under anaërobic conditions. Many forms fail to grow on artificial media; none produce abundant surface growths. Planes of fission generally parallel, producing pairs, or short or long chains." Later (1906, p. 545, and 1908, p. 249) the diagnosis was amended by adding: "never packets. Generally stain by Gram. Produce acid in dextrose and lactose broth. Pigment, if any, white or orange."

The careful work of the Winslows indicates that they have probably secured a logical basis for separation of the genera of the *Coccaceae* into two groups. It is not quite so certain, however, that the genera which they include are all that may be recognized. Perhaps the genus *Siderocapsa* Molisch is sufficiently distinct from the other cocci to require a third group in which to place it.

As has been previously shown (Buchanan 1915, p. 530) the subfamily names *Metacoccaceae* and *Paracoccaceae* are invalid in form, and might well be replaced by the tribal names *Micrococceae* Trevisan and *Streptococceae* Trevisan, with an emended diagnosis.

The three tribes recognized may be differentiated by reference to the following key in which are noted some of the distinguishing characters.

Key to tribes of Coccaceae

I. Not epiphytes, not causing the deposition of iron upon capsules.

A. Commonly parasitic, in some forms saprophytic, usually thriving well under anaërobic conditions, not commonly producing luxuriant growths on artificial media, many forms failing to grow except upon special media. Cells in pairs, chains or irregular masses, never regular packets. Gram-positive, with exception of a few strict parasites. Acid usually formed in dextrose and lactose broth. Pigment white, orange or absent.

Tribe I. *Streptococceae*

B. Saprophytes, or facultative parasites. Usually grow best under aërobic conditions, producing abundant to luxuriant growth on artificial media. Planes of fission often at right angles: Cells aggregated in groups, packets or zoogloeal masses. Usually Gram-negative. Pigment as a rule yellow, red or orange.

Tribe II. *Micrococceae*

II. Epiphytes, usually on leaves and stems of water plants. Iron oxid deposited upon the capsule.....Tribe III. *Siderocapseae*

The following list of generic, subgeneric and pseudogeneric names have been used for various cocci.

Merismopedia Meyen, 1839, p. 67 in part
Sarcina Goodsir, 1842, p. 432
Microhaloa Kützing, 1842, p. 169
Sphaerococcus Kützing, 1843, p. 408
Botryococcus Kützing, 1849, p. 892
Micrococcus Cohn, 1872b, p. 151
Microsphaera Cohn, 1872a, p. 237
Diplococcus Weichselbaum, 1874, p. 5
Ascococcus Billroth, 1874, p. 13
Coccobacteria Billroth, 1874, p. 1
Coccoglia Billroth, 1874, p. 7
Coccos Billroth, 1874, p. 4
Diplococcos Billroth, 1874, p. 5
Gliacococcos Billroth, 1874, p. 5
Megacoccos Billroth, 1874, p. 16
Mesococcos Billroth, 1874, p. 6
Micrococcoglia Billroth, 1874, p. 14
Micrococcos Billroth, 1874, p. 6
Monococcos Billroth, 1874, p. 5

Petalococcos Billroth, 1874, p. 6
Streptococcos Billroth, 1874, p. 10
Streptomicrococcos Billroth, 1874, p. 11
Ascococcus Cohn, 1875, p. 154
Leuconostoc Van Tieghem, 1878, p. 198
Gonococcus Neisser, 1879, p. 497
Chlamydatomus Trevisan, 1879, p. 137
Urosarcina Miquel, 1879, p. 517
Merista Van Tieghem, 1884, p. 1114
Staphylococcus Rosenbach, 1884, p. 19
Streptococcus Rosenbach, 1884, p. 22
Gaffkya Trevisan, 1885, p. 105
Neisseria Trevisan, 1885, p. 105
Arthro-Streptokokkus Hueppe, 1886, p. 145
Askokokkus Hueppe, 1886, p. 145
Hyalococcus Schroeter, 1886, p. 152
Leucocystis Schroeter, 1886, p. 152
Botryomyces Bollinger, 1888, p. 177
Pediococcus Balcke, 1884, p. 183
Pseudodiplococcus Bonome, 1888 p. 321
Haematococcus Babes, 1889, p. 81
Babesia De Toni and Trevisan, 1889, p. 1054
Bollingera De Toni and Trevisan, 1889, p. 10
Perroncitoa Trevisan, 1889, p. 29
Schuetzia Trevisan, 1889, p. 29
Diplokokkus Baumgarten, 1890, p. 127
Tetracoccus Billet, 1890, p. 24
Endostreptokokkus Hueppe, 1891, p. 33
Mikrokokkus Hueppe, 1891, p. 33
Pediokokkus Eisenberg, 1891, p. 25
Sphaerokokkus Eisenberg, 1891, p. 50
Rhodococcus Zopf, 1891, p. 28
Iodococcus Miller, 1892, p. 63
Jodococcus Miller, 1892, p. 63
Makrokokkus Miller, 1892, p. 73
Pyococcus Ludwig, 1892, p. 27
Eucoccus Migula, 1895, p. 16

Euplanococcus Migula, 1895, p. 19
Euplanosarcina Migula, 1895, p. 20
Eusarcina Migula, 1895, p. 18
Planococcus Migula, 1894, p. 236
Planosarcina Migula, 1894, p. 236
Lactococcus Beijerinck, 1901, p. 213
Carphococcus Hohl, 1902, p. 338
Karphococcus Hohl, 1902, p. 338
Pseudosarcine Maze, 1903, p. 887
Mesococcus Smith, 1905, p. 174
Monococcus Smith, 1905, p. 174
Pneumococcus Schmidlechner, 1905, p. 291
Albococcus Winslow and Rogers, 1906, p. 541
Aurococcus Winslow and Rogers, 1906, p. 540
Myxokokkus Gonnermann, 1907, p. 883
Pedioplana Wolff, 1907, p. 9
Lactosarcina Beijerinck, 1908, p. 359
Tetradiplococcus Bartoszewicz and Schwarzwasser, 1908, p. 614
Liquidococcus Jensen, 1909, p. 305
Siderocapsa Molisch, 1909, p. 29
Metacoccus Conn, 1909, p. 12
Solidococcus Jensen, 1909, p. 332.
Sporosarcina Jensen, 1909, p. 340
Diplostreptococcus Lingelsheim, 1912, p. 494
Planomerista Vuillemin, 1913, p. 525
Enterococcus (Thiercelin) Rougentzoff, 1914, p. 648
Macrococcus, Coccus, Meningococcus, Kokkus, Galactococcus, etc. of many authors.

Many of these names may be discarded at once as invalid and not entitled to generic recognition.

The following have been used only as casual designations and are not valid genera: *Gonococcus, Pyococcus, Meningococcus, Galactococcus, Enterococcus.*

The following are the names of growth forms and have never been used in a strict generic sense: *Ascococcos, Coccoglia, Coccos, Diplococcos, Gliacoccos, Megacoccos, Mesococcos, Micrococcoglia,*

Micrococcos, Monococcos, Petalococcos, Streptococcos, Streptomicrococcus, Arthro-Streptokokkus, Tetracoccus, Endostreptococcus, Mesococcus, Pseudosarcina, Monococcus, Metacoccus, Macrococcus.

The following are invalid, usually Germanic, spellings of Latin words: *Askokokkus, Diplokokkus, Mikrokokkus, Pediokokkus, Sphaerokokkus, Jodococcus, Makrokokkus, Karphococcus, Kokkus, Myxokokkus, Haematokokkus.*

The following are names of algal genera in which certain bacteria were earlier placed, and are not true bacterial genera: *Gonium, Merismopedia, Sphaerococcus, Microhaloa, Gloeosphaeria.*

The following are invalid because previously used as generic names for plants not bacteria: *Microsphaera, Merista, Haematococcus, Botryococcus, Urococcus.*

The following are subgeneric designations: *Eucoccus, Euplanococcus, Euplanosarcina, Eusarcina.*

The following names are entitled to serious consideration as possibly valid names for cocci; *Sarcina, Micrococcus, Diplococcus, Ascococcus, Leuconostoc, Chlamydatomus, Staphylococcus, Streptococcus, Gaffkya, Neisseria, Hyalococcus, Leucocystis, Botryomyces, Pediococcus, Pseudodiplococcus, Babesia, Bollingera, Perroncitoa, Schuetzia, Urosarcina, Rhodococcus, Iodococcus, Planococcus, Planosarcina, Lactococcus, Carphococcus, Pneumococcus, Albococcus, Aurococcus, Pedioplana, Lactosarcina, Tetradiplococcus, Liquidococcus, Solidococcus, Sporosarcina, Diplostreptococcus, Planomerista, Siderocapsa.*

Certain genera which have been proposed can scarcely be recognized at the present time, because of insufficient characterization by the authors. Such genera are:

Leucocystis, Chlamydatomus, Ascococcus, Babesia, Iodococcus, Haematococcus.

Tribe I. **Streptococceae** Trevisan, 1889, p. 1051, emended

Synonyms:

Eu-Ascococceae Trevisan, 1889, p. 1035, in part
Gaffkyeae Trevisan, 1889, p. 1042, in part
Paracoccaceae Winslow and Rogers, 1905, p. 1051

Cells spherical when isolated. Usually parasitic, growing well in the absence of oxygen, particularly in the presence of carbohydrates, from which acid is developed. Gas rarely produced. Many forms require special media. Abundant surface growths are rarely developed. Planes of fission are commonly parallel, resulting in the formation of pairs, or of longer or shorter chains of cells; regular packets of cells never formed. With the exception of a few strict parasites, the cells are Gram-positive. Pigment white, orange or none.

In a previous article (Buchanan, 1915, p. 541) it was suggested that the genera in this group to be recognized are *Leuconostoc*, *Streptococcus*, *Neisseria* and *Staphylococcus*.

A study of the characteristics of the genus *Neisseria* has led to the conclusion that it should be divided to form the genera *Neisseria* and *Diplococcus*, the former to include Gram-negative organisms, the latter the Gram-positive.

The following key to the recognized genera gives the most important of the differential characters.

Key to the genera of the Streptococceae

A. Cells occurring normally in chains.
  I. Usually parasitic. Not forming zoogloeal masses in sugar solutions.....Genus 1. *Streptococcus*
  II. Saprophytic, occurring in cane sugar solutions in zoogloeal masses. Genus 2. *Leuconostoc*

B. Cells not occurring usually or characteristically in chains.
  I. Parasitic. Cells in pairs, growth as a rule meagre. No pigment formed.
    a. Gram-positive.....Genus 3. *Diplococcus*
    b. Gram-negative.....Genus 4. *Neisseria*
  II. Cells in irregular groups, usually parasites, growth as a rule good, pigment usually orange or white.....Genus 5. *Staphylococcus*

Genus 1. **Streptococcus** Rosenbach, 1884, p. 22; emended, Winslow and Rogers, 1905, p. 669

Synonyms:

Sphaerococcus Marpmann, 1889, p. 121 not *Sphaerococcus* Agardh, 1821, p. 227

Arthrostreptokokkus Hueppe, 1886, p. 144.

Perroncitoa Trevisan, 1889, p. 1053

Babesia? Trevisan, 1889, p. 29
Schuetzia Trevisan, 1889, p. 1052
Lactococcus Beijerinck, 1901, p. 213
Hypnococcus Bettencourt et al., 1904, p. 55
Myxokokkus Gonnermann, 1907, p. 883 not *Myxococcus*, Thaxter, 1892, p. 403
Melococcus? Amiradzibi, 1907, p. 309
Diplostreptococcus Lingelsheim, 1912, p. 494

Usually parasitic, though able to maintain a saprophytic existence. Cells usually in chains of greater or less length, the elements of the chains sometimes arranged as diplococci, never in packets. As a rule Gram-positive. Growth on surface of usual laboratory media not luxuriant. Growth better in liquid media containing sugars, which are as a rule fermented with acid production, but rarely gas. Usually do not liquefy gelatin or reduce nitrates. No formation of zoogloeal masses in sugar solutions.

The type species is probably *Streptococcus pyogenes* Rosenbach.

Genus 2. **Leuconostoc** Van Tieghem, 1878, p. 198; emended (as *Ascococcus*), Winslow and Rogers, 1905, p. 669

Synonyms:
Ascococcus Cienkowski, 1878, p. 12
not *Ascococcus* Cohn, 1875, p. 154
Leucocystis? Schröter, 1886, p. 152

Saprophytic, usually growing in cane sugar solutions. Cells in chains or pairs. Forming large zoogloeal masses when grown in sugar solutions. Produces acid actively from carbohydrates. Some types at least are Gram-negative.

The type species is *Leuconostoc mesenteroides* (Cienkowski) Van Tieghem.

Genus 3. *Diplococcus* Weichselbaum, 1886, p. 506, emended

Synonyms:
Klebsiella Trevisan, 1885, p. 94 in part
Hyalococcus Schroeter, 1886, p. 152
Pseudodiplococcus Bonome, 1888, p. 321
Pneumococcus? Schmidlechner, 1905, p. 291

Parasites growing poorly or not at all on artificial media. Cells usually in pairs, capsulated. Fermentative powers high, most strains forming acid in dextrose, lactose, saccharose and inulin. The cells are Gram-positive.

The type species is *Diplococcus pneumoniae* Weichselbaum. It is possible that this genus should be combined with *Streptococcus.*

Genus 4. **Neisseria** Trevisan, 1885, p. 105

Synonyms:

Diplococcus Weichselbaum, in part

Parasites, growing poorly in most laboratory media. Cells usually in pairs, flattened, coffee-bean shaped. Gram-negative. As a rule showing considerable fermentative power in carbohydrate media.

The type species is *Neisseria gonorrhoeae* Trevisan.

Genus 5. **Staphylococcus** Rosenbach, 1884, p. 19

Synonyms:

Micrococcus Cohn, 1872 b, p. 151, in part
Botryomyces Bollinger, 1888, p. 177
Botryococcus Kitt, 1888, p. 247
not *Botryococcus* Kützing, 1849, p. 892
Bollingera Trevisan, 1889, p. 1039
Gaffkya Trevisan, 1885, p. 105
Pyococus Ludwig, 1892, p. 27
Carphococcus Hohl, 1902, p. 338
Albococcus Winslow and Rogers, 1906, p. 541
Aurococcus Winslow and Rogers, 1906, p. 205
Liquidococcus Jensen, 1909, p. 332
Indolococcus Jensen, 1909, p. 340
Peptonococcus Jensen, 1909, p. 340
Enterococcus (Thiercelin?) Rougentzoff, 1914, p. 648

Usually parasitic, cells as a rule in irregular groups or short chains, rarely in true packets, usually Gram-positive. Growth fair to good on the surface of artificial media. Sugars as a rule

fermented with acid. Gelatin commonly liquefied. Nitrates may or may not be reduced. Pigment orange or white.

It is probable that the genus should be divided upon the basis of pigment production into two genera, *Staphylococcus* and *Albococcus*, or perhaps these should rank as subgenera.

The type species is *Staphylococcus aureus* Rosenbach.

Tribe II. **Micrococceae** Trevisan, 1889, p. 1067; emended (as *Metacoccaceae*), Winslow and Rogers, 1905, p. 669

Synonyms:

Sarcineae Trevisan, 1889, p. 1044, in part
Metacoccaceae Winslow and Rogers, 1905, p. 669

Saprophytes or facultative parasites. Usually grow best under aërobic conditions. Grow well on artificial media, producing abundant surface growths. Planes of fission often at right angles, cells aggregated in groups, packets or zoogloea masses. Generally Gram-negative. Usually pigmented yellow, orange or red.

The Winslows have included three genera in this group, *Micrococcus*, *Sarcina* and *Rhodococcus*. A key for the quick separation of these genera may be constructed as follows:

Key to the genera of Micrococceae

A. Cells not in regular packets.
  1. Pigment generally yellow.....Genus 1. *Micrococcus*
  2. Pigment red.....Genus 2. *Rhodococcus*
B. Cells in regular packets. Pigment yellow or orange.....Genus 3. *Sarcina*

Genus 1. **Micrococcus** Cohn, 1872, p. 151, emended; Winslow and Rogers, 1905, p. 669

Synonyms:

Microsphaera Cohn, 1872a, p. 151
  not *Microsphaera* Leveille, 1851, p. 381
Ascococcus? Cohn, 1875, p. 154
Pediococcus Balke, 1884, p. 183
Merista Van Tieghem, 1884, p. 1114
  not *Merista* (Banks and Soland) Cunningham, 1839 p. 47
Planococcus Migula, 1894, p. 236

Carphococcus Hohl, 1902, p. 338
Urococcus Miquel, 1888, p. 518
not *Urococcus* Kützing, 1849, p. 206
Pedioplana Wolff, 1907, p. 91
Tetradiplococcus Bartoszewicz and Schwarzwasser, 1908, p. 614
Solidococcus Jensen, 1909, p. 332
Planomerista Vuillemin, 1913, p. 525

Saprophytes or facultative parasites. Cells in plates or in irregular masses (never in long chains or packets). Generally Gram-negative. Growth on agar abundant, with formation usually of yellow pigment. Dextrose broth as a rule slightly acid and lactrose broth neutral. Gelatin frequently liquefied.

The type species is *Micrococcus luteus* (Schroeter) Cohn.

Genus 2. **Rhodococcus** Zopf, 1891, p. 28, emended; Winslow and Rogers, 1906, p. 546

Synonyms:
Not *Rhodococcus* Molisch, 1907, p. 20

Saprophytes. Cells in groups or regular packets. Usually Gram-negative. Abundant growth with red pigment on surface of culture media. Slight acid from dextrose, none from lactose. Gelatin rarely liquefied. Nitrate usually reduced to nitrites, but not to ammonia.

The type species is probably *Rhodococcus roseus* (Flügge) Winslow.

Genus 3. **Sarcina** Goodsir, 1842, p. 432, emended; Winslow and Rogers, 1905, p. 659

Synonyms:
Lactosarcina Beijerinck, 1908, p. 359
Urosarcina Miquel, 1879, p. 517
Tetradiplococcus? Bartoszewicz and Schwarzwasser, 1908, p. 614
Planosarcina Migula, 1894, p. 236
Pseudosarcina? Maze, 1903, p. 887
Sporosarcina Jensen, 1909, p. 340

Saprophytes or facultative parasites. Division occurs under favorable conditions in three planes, producing regular packets. Usually Gram-negative. Growth on agar abundant with formation of yellow pigment. Dextrose broth slightly acid, lactose broth generally neutral. Gelatin frequently liquefied. Nitrates may or may not be reduced.

The type species is *Sarcina ventriculi* Goodsir.

Tribe III. **Siderocapseae** *Trib nov.*

Cells spherical or ovoid, non-motile, epiphytic upon the leaves and other parts of water plants. Have not been cultivated. Thick capsules enclosing the cells become encrusted with iron oxid.

One genus only has been described, *Siderocapsa.*

Genus 1. **Siderocapsa** Molisch, 1909, p. 29

One to many spherical to ovoid small cells embedded in a globule of capsular material, epiphytic on water plants. The species have not been cultivated. Best recognized by staining with Schiff's reagent. Motility not known.

The type species is *Siderocapsa Treubii* Molisch.

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BACTERIUM PYOGENES AND ITS RELATION TO SUPPURATIVE LESIONS IN ANIMALS

By ARCHIBALD R. WARD

Bureau of Animal Industry, Washington, D. C.

Lucet (1893) published a preliminary note on the bacteriology of suppuration in cattle and gave a very brief description of a non-motile rod under the name of *Bacillus pyogenes bovis*. Grips (1898) described under the name of *Bacillus pyogenes suis* an organism frequently found by him associated with suppuration in swine. Künnemann (1903) used the name *Bacillus pyogenes bovis* for an organism found by him frequently in abscesses in cattle. Glage (1903) concluded that the two organisms described by Grips and by Künnemann were identical and proposed the name *Bacillus pyogenes*, or *Bacterium pyogenes*, according to the Migula classification. Probably the organism is identical with the polyarthritis bacillus described by Poels even earlier than the work of Grips. The organism has also been isolated from suppurative lesions in goats and sheep (Olt. 1908; Poels, 1910), and from a number of wild animals in zoölogical gardens.

The present writer has encountered the organism in the hog associated with polyarthritis, in abscesses in the lungs and elsewhere. Lesions of the uterus and udder of the cow have also been observed.

The fact that such a common pyogenic organism of the domesticated animals long escaped discovery by European workers, and still longer has escaped attention of workers in the United States, is a matter susceptible of explanation. It is very small and takes stains irregularly. On account of its pleomorphism, a microscopic examination of pus containing it would ordinarily lead to the conclusion that micrococci, rods, and possibly streptococci were present. However, its characteristic of failing

to grow well on common agar is by far the best explanation of why it escapes isolation. Even when serum agar or solid blood serum is used, cultures may not be obtained, if the amount used for seeding is small, and especially if the abscess is old, in which case most of the organisms have died.

ISOLATION IN PURE CULTURE

The present writer has had success in isolating the organism by the employment of serum agar plates as described by Künnemann (1903). Cattle serum is rendered sterile by filtration through a Berkefeld filter, and is distributed with aseptic precautions in small test tubes, each containing about 5 cc. of serum. Just previous to pouring the plates, the contents of one of these tubes is added to the cooled agar and thus constitutes about one-third of the bulk of the medium. Grips' method of streaking out the suspected material on slanted solidified blood serum also gives good results. Olt (1908) claims to be able by the use of a rabbit to isolate the organism when it is present in the suspected material in very small numbers. The material is injected under the fold of skin on the posterior border of the ear. Under these conditions, saprophytes die out rapidly, while *B. pyogenes* multiplies. After twenty-four to forty-eight hours the area is disinfected, a small amount of pus is squeezed out and cultures on slanted serum are made therefrom. He reports success in the use of the method on such material as scrapings from the soft palate of a cow, and the fluid that exudes on pressure from the tonsil of a pig.

Morphology. The organism is one of the smallest of the bacteria. It varies in size enormously under the different conditions afforded by exudates and the various culture media. The smallest form may appear as a mere visible point and is especially common in old abscesses. The rods vary from 0.3 to 2 microns in length, and are about 0.2 micron broad. In exudate forms resembling the Rotlauf bacillus are seen. Chain formation is not rare. Tapering forms may occur. Spore formation, capsule, pigment production, motility and acid-fast character are absent. Irregularity of staining with carbol fuchsin is

common, but bipolar forms do not occur. The organism is stained readily by the ordinary basic anilin dyes. When stained with alkaline methylene blue, banding is common. Occasionally a long form will be found which displays darker stained areas at the ends, suggestive of *B. diphtheriae*. The Neisser stain does not reveal granules as in *B. diphtheriae*, for the cells are uniformly stained brown. The organism is Gram-positive, a fact that is useful when a microscopic search is made for it in exudate. However, writers have warned that the stay in alcohol should not be too prolonged. Discrepancies among the opinions of the earlier writers as to whether the organism is Gram-positive or not have been harmonized by Olt (1908). He explains that organisms that have been long dead in old exudates are Gram-negative, while live organisms give a Gram-positive reaction. Holth has modified the Gram method for staining tissues as given on p. 626.

Temperature requirements. Berger (1907) sets the range of temperature at which *B. pyogenes* will grow at 24° to 40°, with 37° as the optimum. He finds the thermal death point to lie between 55° and 59°. Milk cultures will remain alive longest at room temperature. The present writer has experienced poor success in keeping cultures alive in the ice box for as long as ten weeks without transplanting, although Berger reports success up to three months.

Conditions for cultivation. The organism is very *dainty* with respect to the culture media upon which it will grow. Serum bouillon, raw serum agar, solidified serum and milk are the most satisfactory media. Solidified blood serum or milk is most valuable for stock cultures. Growth does not occur on potato. Growth on raw serum and common media, such as agar, bouillon and media containing bouillon without the addition of serum, is so slight as not to warrant their use. Growth occurs in gelatin, with liquefaction, but is necessarily slow. The organism grows equally well under aerobic and anaerobic conditions, and in hydrogen as well as in oxygen.

Serum agar. Growth on this medium is detected after thirty-six to forty-eight hours in the incubator as minute points, and

several more days must elapse before maximum growth is reached. European writers describe the sub-surface colonies as having minute thin projections, when observed under weak magnification, giving the colony a thorny appearance. The present writer has observed the condition in the very smallest colonies, but does not find it constant, as might be expected from the great variation of the raw serum content of the medium. Surface colonies rarely exceed 3 mm. in diameter, and are relatively scarce in any plate. With low magnification under the microscope these colonies appear coarsely granular in the center. The granulations are of finer structure peripherally, and near the border the structure appears more uniform. The border line is regular. In optical qualities the colonies are smoky brown by directly transmitted light and bluish white by reflected light.

The subsurface colonies do not frequently assume the ordinary lenticular form, but exhibit a wide variety of shapes, among which trefoil-like forms appear and colonies like the conventionalized heart are observed occasionally. Under low magnification of the microscope the borders of the colonies are rather sharp, but the surface is seen to be slightly irregular. The structure varies in appearance from finely granular with smooth edges, to more coarsely granular with correspondingly rougher edges. Older colonies are surrounded by halo-like areas of cloudiness.

Serum bouillon. The medium becomes clouded by finely flocculent grayish flakes that eventually become deposited as a sediment in the bottom of the tube and to some extent upon the sides, suggesting the behavior of a streptococcus culture. The supernatant fluid may be only slightly clouded or very much so, depending upon the percentage of serum present. The sediment consists of masses of agglutinative bacteria which, when shaken up, produce an irregular clouding of the fluid.

Berger has determined that the more serum the bouillon contains the better the development of the organism and the greater the amount of sediment. The evidence of agglutination in the fluid was found to be greatest when equal parts of serum and of bouillon were employed, and least when 1 part of serum was added to 10 parts of bouillon. He regards this latter propor-

tion as best for a medium to be used for both agglutination work and for animal inoculation.

Slanted, solid cattle serum. European writers describe evidence of growth occurring after several days in the form of pit-like depressions caused by liquefaction. In the experience of the present writer, the liquefaction is more likely to be general over the area seeded, and causes the formation of a trough-like or a shallow irregular depression in the surface of the serum resulting from the gravitation of the liquefied material. Concurrent with this the ordinary collection of fluid at the base of the slant increases in volume. This fluid may be clouded at first but eventually becomes clear with a whitish sediment. As liquefaction progresses the diameter of the mass of solid serum is diminished and the mass is surrounded by a clear fluid, sediment being deposited on the bottom of the tube. In the latest stages the mass of serum often cracks into pieces and eventually the greater part is transformed into clear fluid.

In the experience of the writer the organism frequently fails to grow freely upon solid serum, possibly due to evaporation taking place more rapidly than the liquefaction caused by the organism. The absence of liquefaction in cultures supposed to be *B. pyogenes* should not be given serious consideration until several trials have been made with freshly-prepared medium.

Milk. After forty-eight hours at 37° a slight amount of coagulation induced by development of acid is observed to have occurred in the bottom of the tube, and after three days the coagulation is complete. Separation of the whey occurs, followed by the liquefaction of more or less of the coagulum after a few days. Berger (1907) ascribes the action on the coagulum, as well as the changes induced in gelatin and solid blood serum, to the presence of a proteolytic ferment in the cultures. A culture killed at 59°, when added to solid serum, induced slight liquefaction.

Susceptibility of small animals. The organism has a low degree of pathogenicity for guinea-pigs and mice unless enormous quantities of material are injected. Rats and pigeons are said to be completely immune. The rabbit is available for experimental

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Susceptibility of small animals. The organism has a low degree of pathogenicity for guinea-pigs and mice unless enormous quantities of material are injected. Rats and pigeons are said to be completely immune. The rabbit is available for experimental

inoculation. Subcutaneous injection quite often induces a local abscess. Intra-abdominal inoculation, depending upon the virulence of the organism and the dose, may cause an acute or a chronic peritonitis. The acute purulent peritonitis may cause death in from six to fourteen days, while the chronic form exhibiting adhesions and abscesses may not result fatally for weeks or months. Intravenous inoculation in the rabbit may give negative results or in some cause a pyemia with metastatic foci in various organs including the peritoneum and joints.

The rabbit inoculations made by the writer have resulted in only chronic infections, if any. A rabbit was injected intra-abdominally on November 19 with 3 cc. of a normal salt suspension of growth of *B. pyogenes* from solid serum. The animal died on April 1, autopsy revealing two pyogenes abscesses on the concave surface of the liver. One of these was about the size of a pea, and the other twice as large. The abscesses were united to the adjacent intestine by threads of connective tissue. *B. pyogenes* was isolated from the pus Another rabbit was inoculated intravenously on March 9 with a culture of *B. pyogenes* designated as No. 22. The animal died on September 2. The muscles of the left thigh had been replaced by masses of pus surrounded by very thin transparent walls. The femur had been broken in its lower third, but had reunited. The marrow was red and permeated with channels of pus throughout its whole length. The hip joint was surrounded by a mass of fibrous tissue. *B. pyogenes* was isolated in pure culture.

GENERAL CHARACTER OF LESIONS CAUSED BY BACTERIUM PYOGENES

Bacterium pyogenes differs from the common pyogenic organisms in that it possesses the peculiarity of stimulating the proliferation of connective tissue to form a tumor-like mass similar to granulation tissue, and of subsequently inducing in that tissue the necrotic changes leading to abscess formation. Thus, suppuration caused by *B. pyogenes* in this manner follows a slow chronic course.

In this peculiarity the development of the lesions of *B. pyogenes* bears a general resemblance to the changes occurring in actinomycosis and in tuberculosis. On account of this peculiarity of the organism and of the individuality of the lesions, the changes could more appropriately be classed as a specific infectious disease than among the wound infection suppurative processes. Olt (1904) has suggested the name pyemic cachexia, but pyobacillosis is more generally used.

The earliest stage in an inflammatory lesion is represented by a mass of hard, yellowish colored connective tissue possibly surrounded by a zone of grayish white connective tissue. Later purulent dissolution sets in at the center of the yellowish mass, which gradually becomes transformed into an abscess. Pyogenes pus is generally yellowish in color with a suggestion of green, pasty in consistency, and inodorous.

Holth (1907) has made a most thorough histological investigation of the development of these abscesses. The peripheral part of the small fibrous tumor consists of fibrous connective tissue with very numerous cells. The interior consists of large endothelial-like cells imbedded in a network of connective tissue fibers. These cells are considered as young connective tissue cells which appear weakly granular when stained with strong protoplasmic stains. The nucleus lies either in the center or in the periphery. Similar cells with two nuclei occur often, but multinuclear cells are rare. Among these cells lie imbedded round cells, singly or in groups. The greater proportion of both classes of cells exhibit necrotic changes. In the larger tubercles the isolated groups of round cells become larger and more numerous, especially in the center of the tumor, and here the disintegration of the cells appears. Sections of smaller nodules or of abscess walls, stained for bacteria, show that the larger connective tissue cells may contain enormous numbers of pyogenes bacilli, which are usually short and plump, but which often appear as granules. The round cells contain only a few bacilli. In the degenerated tissue the organisms lie free, the rod shape predominating.

Holth has suggested the following modification of the Gram staining method for use in demonstrating *B. pyogenes:*

The sections are treated one to two minutes with anilin gentian violet. One pours the stain off and puts on the Lügol's solution which is allowed to act one-quarter to one-half minute. Thereupon the sections are immersed in absolute alcohol in which they remain until completely dehydrated. The alcohol is removed with the help of xylol, and the sections then have a bluish black appearance. Then oil of cloves is allowed to act about a minute, after which differentiation is accomplished in a mixture of 1 part of absolute alcohol and 4 parts of xylol, especially to remove the adhering stain and oil of cloves. In closing, pure xylol and balsam are applied.

In this method the bacteria are stained bluish black and the tissue pale reddish color. In objects fixed in formalin the red corpuscles and the elastic tissue take the stain very strongly. They show then a more or less bluish appearance.

The organism is also capable of inducing pure exudative changes in tissue, in inflammations following a chronic course. The exudate is usually a thin slimy fluid, having a whitish yellow, gray, or reddish yellow color. It may be sero-purulent, hemorrhagic or purulent, and generally contains considerable fibrin.

The infection very frequently spreads by metastasis along the blood and lymph streams.

Lesions frequently are found to contain *B. pyogenes* alone, but a wide variety of other suppurative organisms may be associated with it.

A review of the literature of *B. pyogenes* from European sources leads to the conclusion that it plays a most important rôle in animal diseases. In this place I shall no more than attempt to point out the breadth of the field of its activities in animal pathology.

Abscess formation in swine induced by this organism may occur in the widest variety of locations, in the subcutis, the musculature and the skeleton. Likewise, the peritoneum and visceral organs are subject to attack. A somewhat extensive literature has grown up about its relation to lung lesions alone. Here its importance equals, if it does not exceed, that of the swine plague organism. Grips, Glage and Nieberle (1904) state that *B.*

pyogenes may be recognized in the lungs of every case of swine plague. Pütz (1904) found it in 55 per cent of all cases, and Holth (1907) is of the opinion that 55 per cent is too low. It is stated that in old pneumonias *B. suisepticus* may be found, but very frequently it may be shown that this organism is only slightly virulent for experimental animals. Therefore, such organisms are indistinguishable from strains morphologically and biologically identical, which frequently exist in the bronchial contents under normal conditions. *B. pyogenes* seems to be a normal inhabitant of the mouth and of the tonsilar crypts. The intestinal mucosa is often the site of purulent catarrh induced by this organism.

In cattle as in swine *B. pyogenes* causes suppuration in a great variety of locations. Künnemann (1903) has isolated it from 90 per cent of specimens of bovine abscess pus. In 35 per cent of cases he found the organism alone in pus, in 55 per cent in company with other bacteria. It was found associated with other organisms almost uniformly in metritis, mammitis and navel infection. Pneumonia similar in appearance to tubercular pneumonia is caused by *B. pyogenes* in cattle and has been studied by Berger (1908). Poels has made extensive studies of arthritis (1912) and mammitis (1910) caused by this organism.

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INFLUENCE OF HYDROGEN-ION CONCENTRATION OF MEDIUM ON THE REPRODUCTION OF ALFALFA BACTERIA

E. B. FRED AND N. E. LOOMIS

Department of Bacteriology, University of Wisconsin

The value of the hydrogen electrode in determining the reaction of culture media has been shown by Michaelis (1914), Clark (1915, 1917), and others. Their results indicate that bacterial processes are influenced to a marked degree by the hydrogen-ion concentration of the medium. It appears that certain species of organisms are very sensitive to slight changes, while others will develop in a medium having considerable variation in reaction.

The question arises whether the reaction of a medium within certain limits exerts an important influence on the growth of legume bacteria. In this laboratory an attempt has been made to study the effect of reaction on the growth of *B. radicicola* from alfalfa.

METHODS

Mannitol solution was used as a culture medium.

Mannitol	10.0 grams
Magnesium sulfate	0.2 gram
Monobasic potassium phosphate	0.2 gram
Sodium chloride	0.2 gram
Calcium sulphate	0.1 gram
Distilled water	1000.0 cc.

The phosphate was dissolved separately in a little water and the solution made neutral to phenolphthalein with N/1 sodium hydroxid. After sterilizing, the culture medium was again adjusted to the neutral point of phenolphthalein.

Hydrogen-ion concentrations were measured electrometrically. A modification of the apparatus used and described in detail by Loomis and Acree (1911) was used. All readings were made at 25°C. and are expressed in terms of the symbol P_H,

(Sørensen (1909)) representing the log I/C_H. Corrections were made for hydrogen pressure and for variations of the calomel electrodes, as suggested by Loomis and Acree (1916).

REACTION TO PHENOLPHTHALEIN AND HYDROGEN-ION CONCENTRATION

Twenty-four 100 cc. portions of mannitol solution in 750 cc. Erlenmeyer flasks were inoculated with alfalfa bacteria. The cultures were then arranged in pairs and the reaction changed by the addition of sterile N/10 sulphuric acid or sodium hydroxid. Column 2 of table 1 shows the variations in reaction. Immediately after inoculation the hydrogen-ion concentration was determined, and again after two weeks. (See chart 1.) In connection with the measurements of reaction, plate counts of the total number of bacteria were made after two and four weeks. The averages of triplicate plates, and the hydrogen-ion concentration, as well as the titrated acidity or alkalinity are given in table 1.

TABLE 1

Reaction and growth of legume bacteria

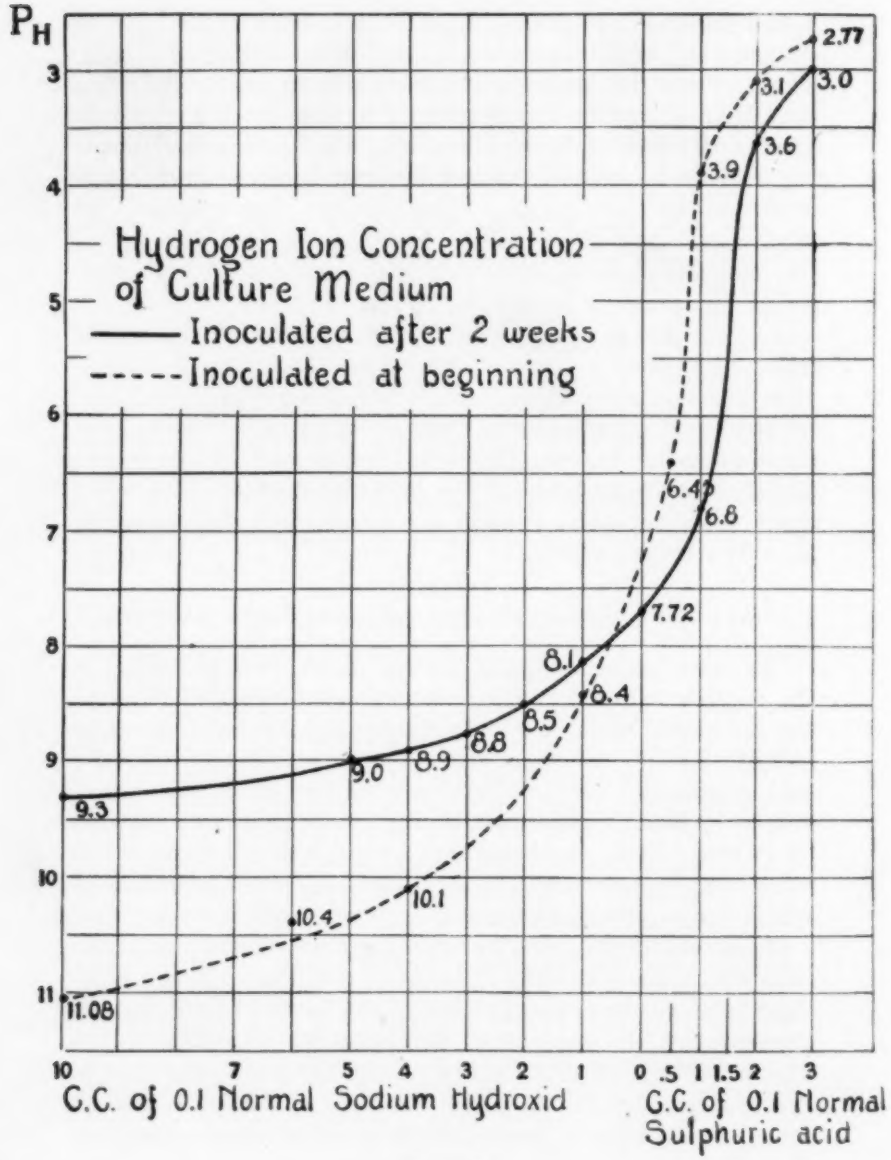
Flask number	Normal acid or alkali in 100 cc. of medium	P_H			Bacteria in 1 cc. of culture medium			
		Beginning		After two weeks	After two weeks		After four weeks	
	Cubic centimeter	Theory in water	Found		Total	Relative	Total	Relative
			In culture medium					
1	0.3 H_2SO_4	2.52	2.77	3.00	No growth		No growth	
2	0.2 H_2SO_4	2.70	3.10	3.60	No growth		No growth	
3	0.1 H_2SO_4	3.00	3.90	6.80	19,580,000	75	31,000,000	58
4	0.05 H_2SO_4	3.30	6.45		21,520,000	83	32,500,000	61
5	Neutral	6.86	7.2	7.72	25,900,000	100	53,200,000	100
6	0.1 NaOH	10.72	8.4	8.1	20,280,000	78	29,200,000	55
7	0.2 NaOH	11.02		8.5	18,280,000	70	30,100,000	56
8	0.3 NaOH	11.20		8.8				
9	0.4 NaOH	11.32	10.1	8.9	13,220,000	51	29,800,000	56
10	0.5 NaOH	11.42		9.0				
11	0.7 NaOH	11.57	10.4		14,540,000	56	21,900,000	41
12	1.0 NaOH	11.72	11.1	9.3	12,100,000	46	11,600,000	21

The results show clearly the difference between the concentration of hydrogen ions and the total concentration of acid or alkali. From the hydrogen electrode measurements, it is evident that change in the hydrogen-ion concentration is much greater with a given increase in acidity than with a corresponding increase in akalinity, especially after bacterial growth has continued for two weeks. Consequently large amounts of alkali have little effect on the hydrogen-ion concentration of the mannitol medium, while even small amounts of acid cause a decided change in true acidity. The difference in the P_H for a given amount of acid and alkali in water solutions and in the mannitol medium is very noticeable from the figures of table 1. Here the vertical column 3 gives the theoretical P_H in water solutions and columns 4 and 5 the P_H found in the culture medium when inoculated. The buffer action may be due to the mannitol or to products of the bacterial growth. The latter seems more probable, because it is so much more noticeable after two weeks' growth.

HYDROGEN-ION CONCENTRATION AND GROWTH OF BACTERIA

The most important point shown by the data of table 1 is the relation between hydrogen-ion concentration and development of alfalfa bacteria. Plate counts after two and four weeks indicate that *B. radicicola* is much more sensitive to sulphuric acid in mannitol solution than to gram equivalent amounts of sodium hydroxid. Somewhat similar results have been reported by Prucha, (1915), who found that normal hydrochloric acid was much more injurious to the multiplication of *B. radicicola* of alfalfa than equivalent amounts of normal sodium hydroxid.

It was assumed in previous investigations that *B. radicicola* from alfalfa was especially resistant to alkali and likewise especially sensitive to acid. In view of the curve of hydrogen-ion concentration, it seems that the apparent resistance of the legume bacteria to alkali is due to the slight concentration of hydroxyl-ions in the mannitol solution. From the data of table 1 it will be seen that alfalfa bacteria in mannitol solution


PH
3
4
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10
11
Hydrogen Ion Concentration of Culture Medium
Inoculated after 2 weeks
Inoculated at beginning
2.77
3.1
3.0
3.6
3.9
6.4
6.8
7.72
8.1
8.4
8.5
8.8
8.9
9.0
9.3
10.4
10.1
11.08
10 7 5 4 3 2 1 0 .5 1 1.5 2 3
C.C. of 0.1 Normal Sodium Hydroxid
C.C. of 0.1 Normal Sulphuric acid

CHART 1

gave the highest count in a neutral solution, although small amounts of alkali had very little effect on the number of bacteria. On the other hand, acid in gram equivalent amounts seriously retarded or prohibited growth. The relation of growth to hydrogen-ion concentration remained almost the same after two and four weeks.

EFFECT OF BACILLUS RADICICOLA ON HYDROGEN-ION CONCENTRATION

Chart 1 gives the curves of the hydrogen-ion concentration of mannitol solution before and after the growth of legume bacteria. The hydrogen-ion concentration is plotted in terms of P_H on the ordinate and the cubic centimeters of acid or alkali on the abscissa. It should be remembered that as P_H increases the concentration of hydrogen-ions decreases and *vice versa*. One of the most striking facts noted by these curves is the difference in the hydrogen-ion concentration of the culture media before and after growth of bacteria. It will be seen that this change is most marked in the case of high P_H of the medium. Apparently the alfalfa bacteria bring about changes in the reaction of the medium which are favorable for their reproduction. The growth of different species of legume bacteria in culture media of varying hydrogen-ion concentration will be discussed in a future paper.

The authors are indebted to Dr. S. F. Acree for many helpful suggestions.

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THE COLON-AEROGENES GROUP FROM SILAGE

O. W. HUNTER

From the Bacteriological Laboratory, Kansas Agricultural Experiment Station

A study of silage fermentation has demonstrated the *colon* organisms to be a predominating type in the earlier stages of ripening. Analyses made from many samples of alfalfa, cane, corn and kafir forage, at time of filling the silo, have given counts ranging from 1000 to 1,000,000 of these organisms per gram of forage. Examinations made from different forage collected in the fields under aseptic conditions showed their presence in numbers ranging from 1000 to 100,000 organisms per gram. This demonstrates that the common forage crops are natural hosts for the colon-aerogenes group, and the large numbers found on the forage as it enters the silo are not due to contamination through handling and cutting, but represent, in a majority of cases, the actual numbers living on the forage.

Numerous investigators have reported the presence of the colon-group on grains, grasses and flowers. They have not agreed, however, as to whether the presence of such organisms signifies fecal contamination or represents a specific type characteristic to grains.

Rogers, Clark and Evans (1915) have examined 166 cultures of coli-like bacteria obtained from dried grains, and demonstrated that none of the types agree with the characteristic flora of the bovine species. Previous to the work of Rogers and his associates, Burri and Andrejew (1910) mentioned that the colon-aerogenes types appearing on grass could be differentiated from the intestinal types. Prescott (1902, 1906) stated that the colon group was able to grow and reproduce on grain, and that there was no distinction between the grain and fecal types.

Preliminary investigations in this laboratory indicate that the colon-aerogenes group commonly present on forage finds there

a favorable environment for growth. Alfalfa was grown in the laboratory where all sources of contamination were prevented except from the soil. The bacteriological examination of the forage gave a count of over 100,000 coli-like organisms per gram. The only possible source of infection from such organisms was through the root system, which does not seem a probable source, or through contamination from the soil as the plants broke through the surface. This latter source is no doubt important.

The purpose of this experiment was to study the physiological activities of the organisms of the colon-aerogenes group present in silage.

METHOD OF PROCEDURE

The number of cultures studied was 110, isolated from the following sources; 95 from various kinds of silage and at different stages of fermentation, of which 53 were from alfalfa silage, 39 kafir silage, and 3 from corn silage. In addition to these 15 strains were obtained from alfalfa and kafir forage which was collected from the fields under conditions as nearly aseptic as possible. Lactose bile in fermentation tubes was used as an enrichment medium. The tubes showing gas were plated on plain and glucose agar, and agar slants were inoculated from representative colonies picked from the plates. All cultures were classified into MacConkey's four groups, according to gas production in glucose, lactose, sucrose, and dulcite broth.

The hydrogen-ion concentration was determined by the method suggested by Clark and Lubs (1915) using methyl red as an indicator. According to their results all grain cultures exhibited a low hydrogen-ion concentration, while that of the fecal strains was high.

The Voges-Proskauer reaction was observed in the medium used for making the hydrogen-ion determination. All cultures were studied as to motility, and action in litmus milk and gelatin.

The temperature of incubation was 37.5°C. for all cultures. The gas production and action in litmus milk were noted after two days, the Voges-Proskauer reaction and hydrogen-ion concentration after four days and the action on gelatin after ten

days' incubation. The gelatin cultures at the end of the incubation period were placed in ice water for several minutes. The failure of the tubes to solidify indicated liquefaction. All cultures showing gelatin liquefaction and absence of gas in lactose were discarded. Motility was observed in twelve hour broth cultures.

EXPERIMENTAL DATA

Table 1 contains a complete summary of the biochemical characteristics of all cultures studied.

TABLE 1

A summary of the physiological activities of the colon aerogenes group from silage

GROUP	TOTAL NUMBER	PER CENT MOTILE	PER CENT ACID TO METHYL RED	PER CENT ALKALINE TO METHYL RED	PER CENT SHOWING VOGES-PROSKAUER REACTION	PER CENT COAGULATING LITMUS MILK
B. coli-communis	12	100.0	100	0	0	100
B. coli-communior	34	55.8	0	100	100	3
B. (lactis) aerogenes	53	64.1	0	100	100	0
B. acidi (lactici)	11	90.9	100	0	0	81.8

The cultures have been classified according to MacConkey's four principal groups, as represented by *B. coli-communis*, *B. coli-communior* *B. (lactis) aerogenes* and *B. acidi (lactici)*.

All cultures of the *B. coli-communis* and *B. acidi (lactici)* groups gave a high hydrogen-ion concentration, which indicates fecal origin, while all cultures of the *B. coli-communior* and *B. (lactis) aerogenes* groups gave a low hydrogen-ion concentration, which signifies grain origin. More recent results obtained, *at this laboratory*, from the study of the colon group from other sources do not, correspond in this respect, however. Neither is there agreement with Greenfield's (1916) work on the colon organisms isolated from water and ice. She found the organisms exhibiting a high and low hydrogen-ion concentration to be distributed in all of MacConkey's four groups.

Browne (1915) states that *B. coli-communis* and *B. acidi (lactici)* are the predominating groups in fresh human feces.

An average of 11 samples gave 31.6 per cent *B. coli-communis*, 57.2 per cent *B. acidi* (*lactici*), 8.3 per cent *B. coli-communior* and 3 per cent *B.* (*lactis*) *aerogenes*. Likewise an average of the 432 cultures isolated by Greenfield from water and ice indicates that 96.3 per cent of the *B. coli-communis* and 91.3 per cent of the *B. acidi* (*lactici*) groups are of fecal origin, while 75.3 per cent of the *B. coli-communior*, and 21 per cent of the *B.* (*lactis*) *aerogenes* groups are of grain origin.

While the figures cited tend to show that the majority of the fecal strains are represented by the *B. coli* (*communis*) and *B. acidi* (*lactici*) groups, not all investigators are in accord.

According to MacConkey's classification the results indicate that 10.9 per cent of the organisms studied were *B. coli-communis* 30.9 per cent *B. coli-communior*, 48.18 per cent *B. lactis* (*aerogenes*) and 10 per cent *B. acidi* (*lactici*). Classified according to origin 79.08 per cent were non-fecal strains, while 20.9 per cent were fecal types.

A direct correlation can be noted between the Voges-Proskauer reaction and hydrogen-ion concentration, which agrees with the results of Levine (1916) and Greenfield. All cultures having a high hydrogen-ion concentration gave a negative Voges-Proskauer reaction, while the converse was true for those cultures having a low hydrogen-ion concentration.

In litmus milk 90.9 per cent of all fecal strains produced acid and coagulation, while 98.5 per cent of the non-fecal types showed only an acid reaction.

While motility of all cultures was determined little importance is attributed to it as a means of classification.

CONCLUSION

1. The study of 95 coli-like cultures isolated from different kinds of silage, and 15 strains obtained from the growing fields of alfalfa and kafir, demonstrates that 48.18 per cent of the organisms are *B.* (*lactis*) *aerogenes*, 30.9 per cent *B. coli-communior*, 10.9 per cent *B. coli-communis* and 10 per cent *B. acidi* (*lactici*).

2. Classified according to origin, as differentiated by methyl red, 79.08 per cent were non-fecal strains, while 20.9 per cent were of fecal origin.

3. All the strains represented by the *B. coli-communis* and *B. acidi* (*lactici*) groups were fecal types, while the organisms included in the groups represented by *B. coli-communior* and *B.* (*lactis*) *aerogenes* were non-fecal strains.

4. A correlation between the Voges-Proskauer reaction and the hydrogen-ion concentration was observed in all cultures.

5. Litmus milk was coagulated by 90.9 per cent of all fecal strains, while 98.5 per cent of the non-fecal types exhibited only an acid reaction.

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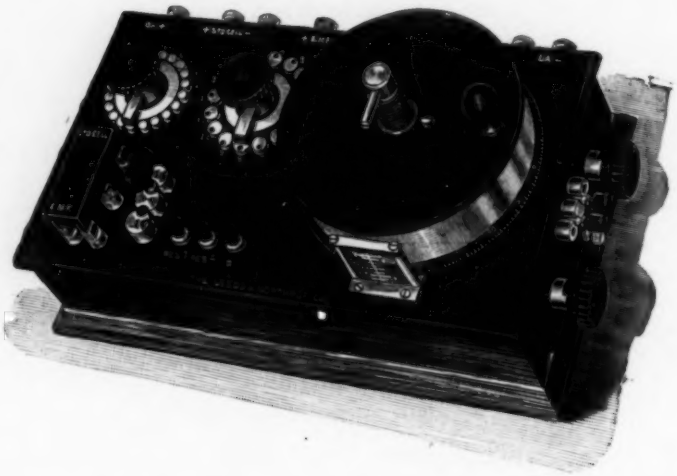

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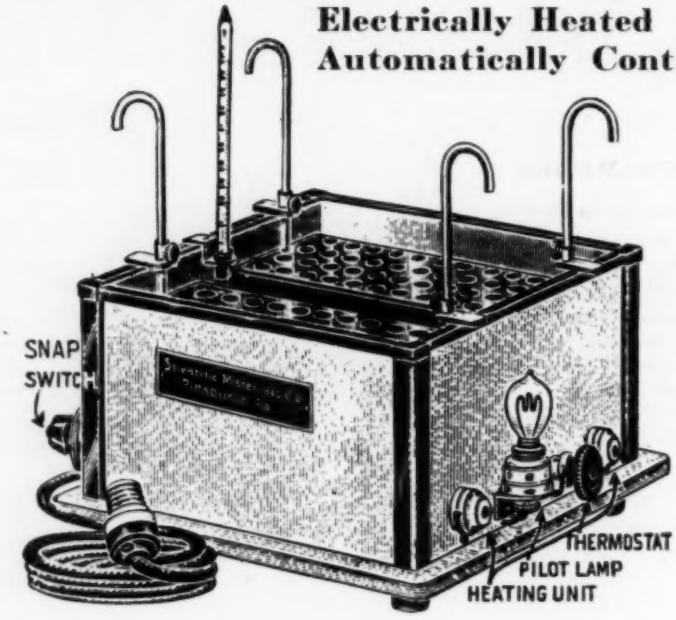

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